



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Veterinary
and Food Administration

Highly Pathogenic Avian Influenza H5N1 in Denmark, Spring 2006

Report



Danish Veterinary and Food Administration
2007

For further information on the Avian Influenza outbreaks in Denmark 2006:

www.foedevarestyrelsen.dk

Postal address: Danish Veterinary and Food Administration
Mørkhøj Bygade 19
DK- 2860 Søborg
Denmark

Preben Willeberg, Chief Veterinary Officer

Tel: +45 33 956119
Tel: +45 33 956115
E-mail: pw@fvst.dk

Helene Rugbjerg, Veterinary Officer

Tel: +45 33 956319
E-mail: hru@fvst.dk

Michael Baumgarten, International Secretariat

Tel: +45 33 956243
E-Mail: mbg@fvst.dk

Cover Page:

The photo was taken in a farm where LPAI H5N2 were detected on 2nd June 2006 in mallard ducklings for restocking of game bird populations. The mallard ducklings did not show any clinical signs of disease.

Printed: Copenhagen, March 2007

Highly Pathogenic Avian Influenza H5N1 in Denmark,
Spring 2006

1. SUMMARY	4
2. BACKGROUND.....	5
3. EPIDEMIOLOGY	6
3.1 DESCRIPTION OF THE EPIDEMIC IN WILD BIRDS	6
3.1.1. SURVEILLANCE OF DEAD WILD BIRDS.....	6
3.1.2.SURVEILLANCE OF LIVE WILD BIRDS	11
3.2 DESCRIPTION OF THE OUTBREAK IN A BACK-YARD HERD.....	12
3.3 DESCRIPTION OF THE SPREAD OF THE DISEASE.....	15
3.4 SURVEILLANCE OF POULTRY AND GAME BIRDS FOR RESTOCKING	18
3.5 SEROLOGICAL METHODS AND EXAMINATION.....	20
3.6 VIROLOGICAL METHODS AND EXAMINATION.....	21
4. DISEASE CONTROL	22
4.1 LEGISLATIVE MATTERS.....	22
4.2 CRISES MANAGEMENT STRUCTURE.....	22
4.3 MEASURES IN CASE OF SUSPICION	23
4.4 MEASURES IN CASE OF CONFIRMED OUTBREAKS	23
4.5 KILLING	23
4.6 CLEANING AND DISINFECTION	24
4.7 MEASURES TAKEN IN PROTECTION AND SURVEILLANCE ZONES.....	24
4.8 REGISTRATION OF POULTRY FLOCKS	24
4.9 INSPECTIONS AND SCREENING IN PROTECTION AND SURVEILLANCE ZONES	25
4.10 SCREENING RESULTS	26
5. CONCLUSIONS.....	27
REFERENCES.....	27
APPENDIX I	28
APPENDIX II.....	32
APPENDIX III	34

1. Summary

A total of 44 wild birds were found infected with Highly Pathogenic Avian Influenza (HPAI) H5N1 in Denmark from the 14 March till the 29 May 2006. During this period, a total of 1120 wild birds found dead and 10 other animals – mainly cats have been examined. Furthermore, 1112 droppings from live wild birds have been examined from the middle of January till the end of June. These wild birds originated from the whole country, but infected birds were found in areas, where the occurrence of wild birds normally are dense in winter/early spring, i.e. mainly the coastal line towards the Baltic sea and shallow areas in the inner Danish waters. Wild birds infected were primarily ducks (60%), but also swans (16%), birds of prey (18%), crows (2%) and other waterfowl (4%) were found positive for H5N1. In all cases, protection and surveillance zones were immediately established around the place of finding on the basis of positive PCR results according to the Commissions Decision 2006/115/EC.

Further to this, a back-yard flock was found infected with HPAI H5N1 on 18 May 2006. The source of infection was probably wild birds, because the affected birds had not been kept in net-covered enclosures in order to minimize contact with wild birds as prescribed in the Danish legislation at that time, and the flock was close to the finding place of a wild bird isolate made a few weeks before. A magpie found dead on the affected premises diagnosed with HPAI H5N1 on the 29 May 2006 supported this. Protection and surveillance zones as well as A and B areas were immediately established on 18 May 2006 on the basis of the positive PCR result according to the Commissions Decision 2006/135/EC. The zones were lifted on 30 June 2006 following an extended screening of poultry within the zones.

2. Background

Highly pathogenic avian influenza (HPAI) is classified as a disease notifiable to the OIE and is causing extremely high mortality in different poultry species. HPAI is caused by an orthomyxovirus of type A. Type A virus is categorised according to its H and N antigens. In total, 16 H and 9 N types have been identified, which gives more than 140 combinations. Most of these have been isolated from birds.

Avian Influenza is found in a HP and a low pathogenic (LP) form. HPAI has always been found among the subtypes H5 and H7. The main clinical signs of HPAI are egg drop, diarrhoea, respiratory disorders and high mortality. Wild birds, especially ducks, are likely being the reservoir for Avian Influenza, and some of these species do not show clinical signs of the disease. They therefore act as silent carriers of infection, which enables transmission to other birds (WHO, 2005; EFSA, 2005).

AI H5N1 can infect cats, seals, whales, minks and polecats/ferrets, but there have only been very few documented cases, although there have been good possibilities of infectious contact. The virus has a zoonotic potential for spread to humans. Worldwide 263 humans have become infected with H5N1, and 157 of these have died from the infection (WHO, 12 March 2007). All cases have been in Asia and the Middle East area following close contact with infected birds. AI has a potential to mutate to a pandemic virus. Hidden infection in birds increases the risk of spread to humans, because there are no warning signals, which can prevent risky behaviour (WHO, 2005).

LPAI can circulate among poultry without giving any symptoms, after which it can mutate and become HPAI. In spring of 2006, HPAI has been found in domestic poultry in commercial flocks in France, Germany and Hungary, in game birds (mallards) for restocking in Sweden and in a backyard flock in Denmark. In Europe, outbreaks of HPAI have previously been observed in 1999, 2000 and 2004 in Italy and in 2003 in the Netherlands (H7).

More information on Avian Influenza in Denmark can be found on the webpage www.foedevarestyrelsen.dk.

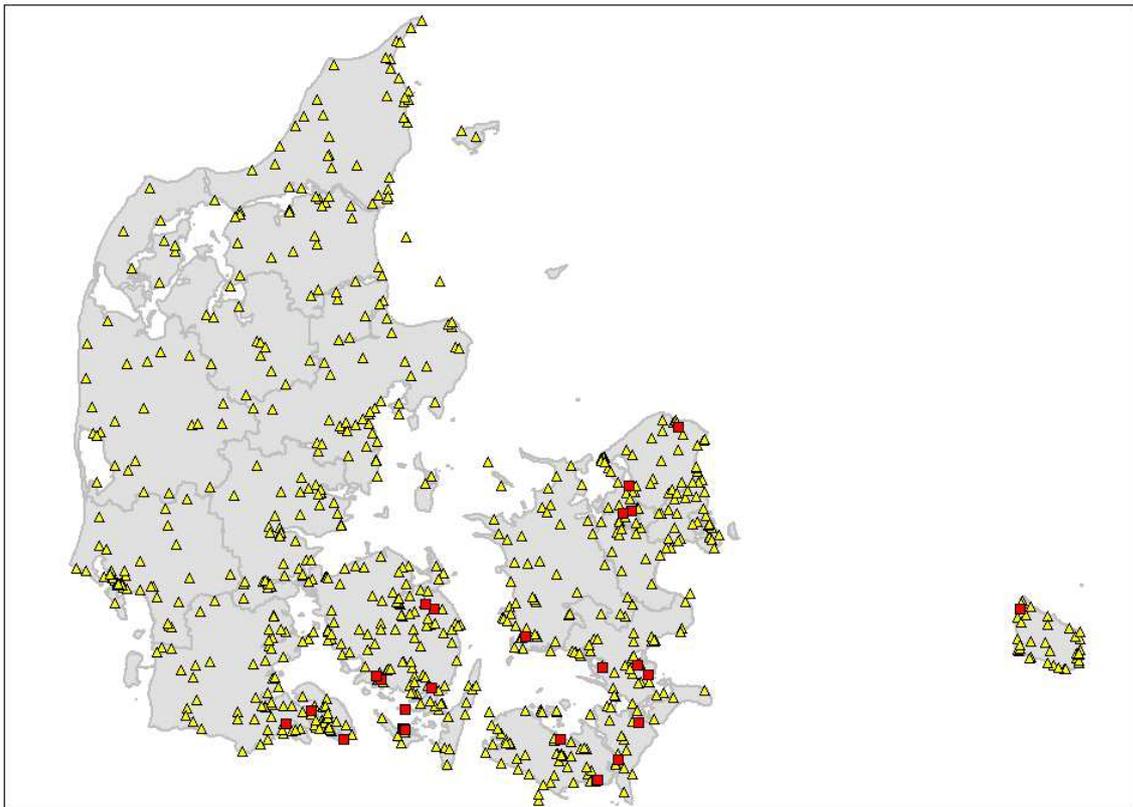
3. Epidemiology

3.1 Description of the epidemic in wild birds

3.1.1. Surveillance of dead wild birds

The infection with HPAI H5N1 in wild birds spread from Asia to the European continent in the middle of January 2006. After the infection with HPAI H5N1 was detected on 14 February 2006 in wild birds on the Island of Rügen in Germany very close to the Danish border, the Danish Veterinary and Food Administration announced a call to the general public for reporting the finding of dead wild birds. The birds were under appropriate safety measures collected by personnel from the Danish Emergency Management Agency and brought to the laboratory for virological examination. In total, 1120 dead birds and 10 other animals – mainly cats – were examined during the first six months of 2006. The Danish Veterinary and Food Administration decided that only findings of five or more dead or sick waterfowl in wetlands, one or more dead or sick birds of prey, two or more dead or sick crows in wetlands or five or more dead or sick birds on the same locality outside wetlands were to be examined. The dead wild birds examined were from the whole country as illustrated on Figure 1.

Figure 1
Wild birds testing positive and negative from January to May 2006. Yellow triangles are H5N1 negative and red squares are H5N1 positive birds.

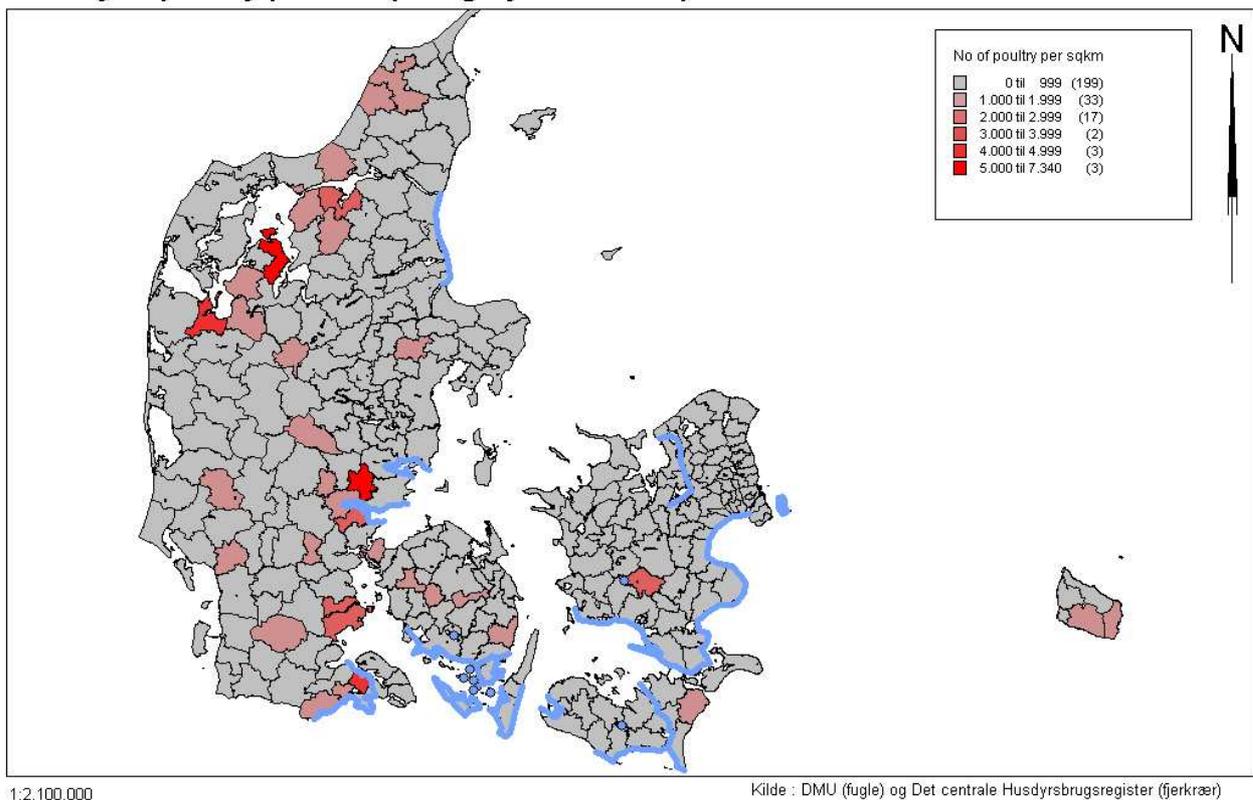


Comparing Figure 1 with Figure 2 illustrates, that birds infected with HPAI H5N1 have been found in areas, where the density of wild ducks and swans normally are high in winter/early spring.

Figure 2

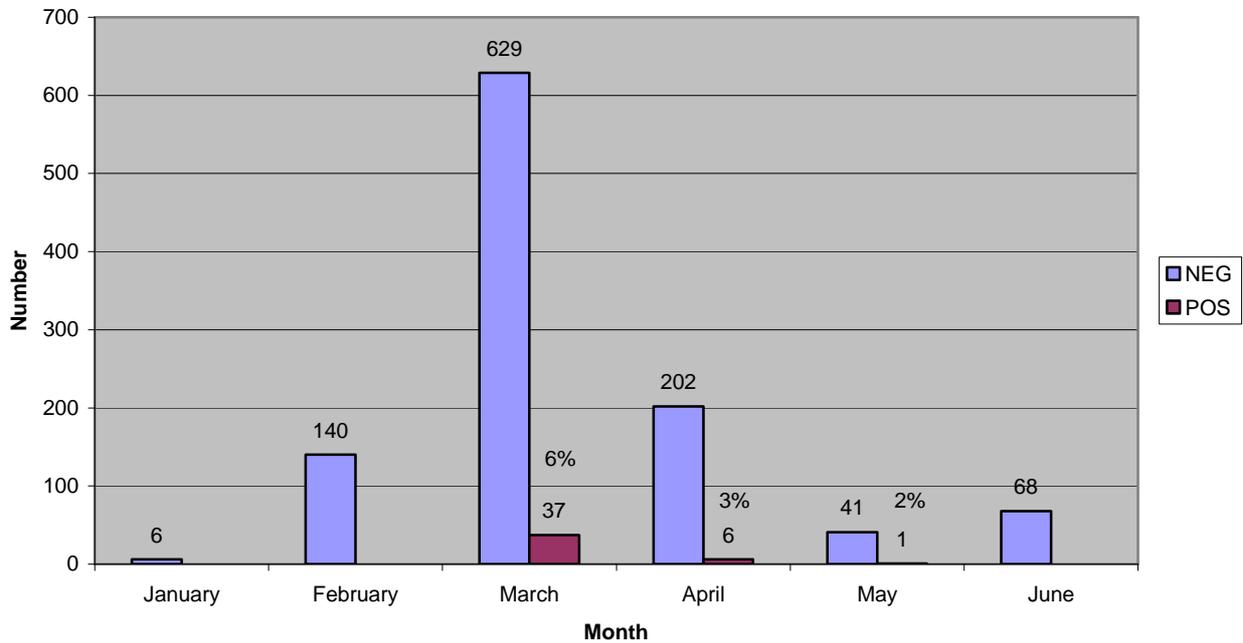
Blue lines indicate important wintering areas for tufted ducks, pochards, scaup ducks and mute swans expected in Denmark. High density of poultry is shown in red shades (25 March 2006).

**Resting and wintering areas for tufted ducks and swans in DK (blue line)
Density of poultry per km² (red /grey color code)**



In January and the beginning of February only very few dead wild birds were observed and sent in for examination, but after the infection spread among wild birds in Northern Europe from the middle of February this changed considerably (Figure 3). The number of birds examined peaked in March declining through May and June. The figures also illustrates, that many wild birds died during the winter, which was colder and lasted longer than normally. The first finding of HPAI H5 in a wild bird – a common buzzard - in Denmark was detected on 14 March 2006. Subsequently, it was shown to be of the type H5N1.

Figure 3
Monthly distribution of the number of dead birds examined from January to June 2006.



The findings of 44 wild birds infected with HPAI H5N1 occurred in six counties in Denmark during March-May of 2006. The first case was found on 14 March 2006 and the last case was confirmed on 29 May 2006. During this period the virus was circulating in the wild bird population, but there was only one parallel finding among poultry, indicating that this epidemic was not caused by a continuing spill-over from poultry like suspected in Asia.

As illustrated in Table 1 and on Figure 4, the findings of HPAI H5N1 in wild birds were concentrated in the coastal areas around the Baltic Sea and in the shallow areas in the inner Danish waters.

Table 1
Location of cases of HPAI H5N1 in wild birds in 2006

County	Number of infected birds	Number of dead birds tested	Proportional mortality rates
Nordjylland	0	74	-
Viborg	0	21	-
Århus	0	52	-
Ringkøbing	0	25	-
Vejle	0	46	-
Ribe	0	47	-
Storstrøm	8	93	9%
Fyn	27	141	19%
Frederiksborg	4	58	7%
Sønderjylland	3	170	2%
Vestsjælland	1	67	2%
Roskilde	0	21	-
Københavns/Frb. kommune	0	57	-
Bornholm	1	97	1%
Without exact coordinates	0	151	-
Total	44	1120	4%

Figure 4
Zones established as of 7 April 2006 at the height of the epidemic in wild birds

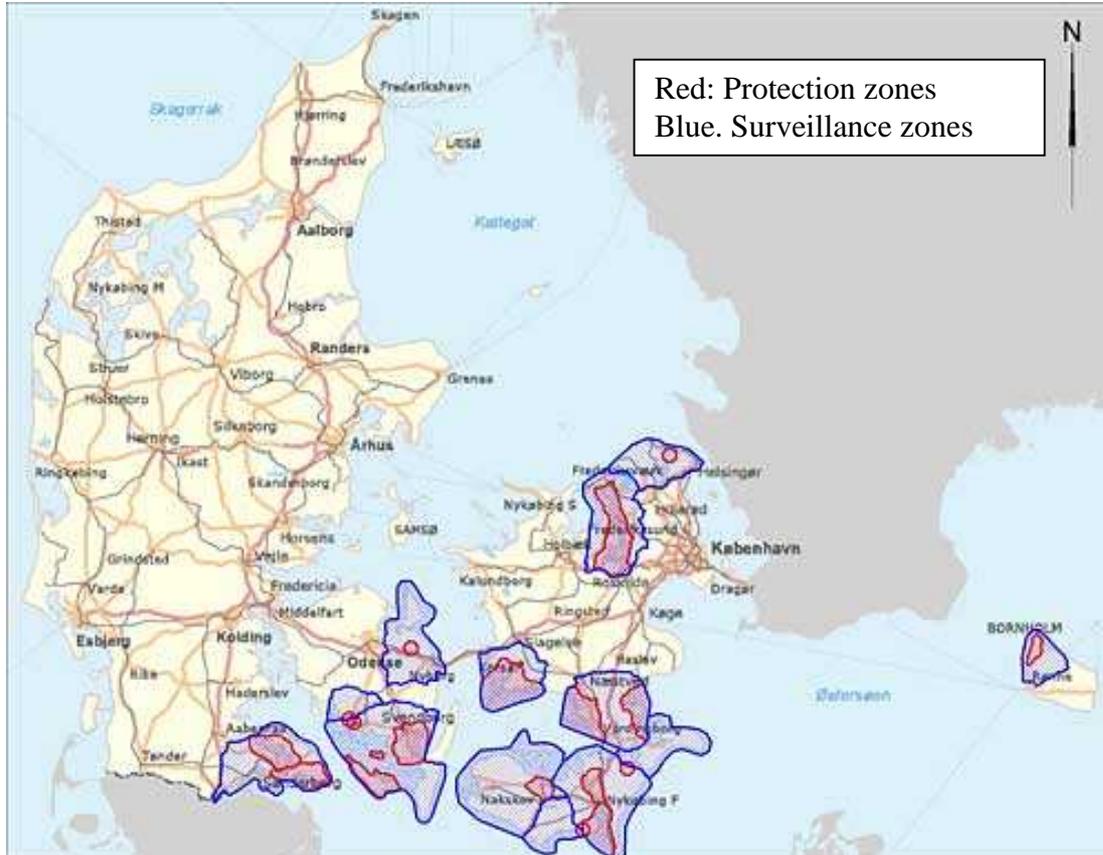


Table 2 illustrates the different species of birds, which were found positive for HP H5N1. In spring 2006, HP H5N1 was most prevalent among ducks followed by birds of prey, swans, crows and other waterfowl.

Table 2
Cases of HPAI in wild birds in 2006 distributed by species groups. Distribution by species is shown in Appendix I.

Group	Number of infected birds	Number of dead birds tested	Proportional mortality rates ¹
Ducks	26	170	15%
Swans	7	181	4%
Birds of prey	8	415	2%
Gulls and terns	0	119	-
Crows	1	60	2%
Other water fowl	2	110	2%
Other birds	0	65	-
Total	44	1120	4%

Figure 5
Number of dead birds examined from January to June 2006 distributed by species groups.

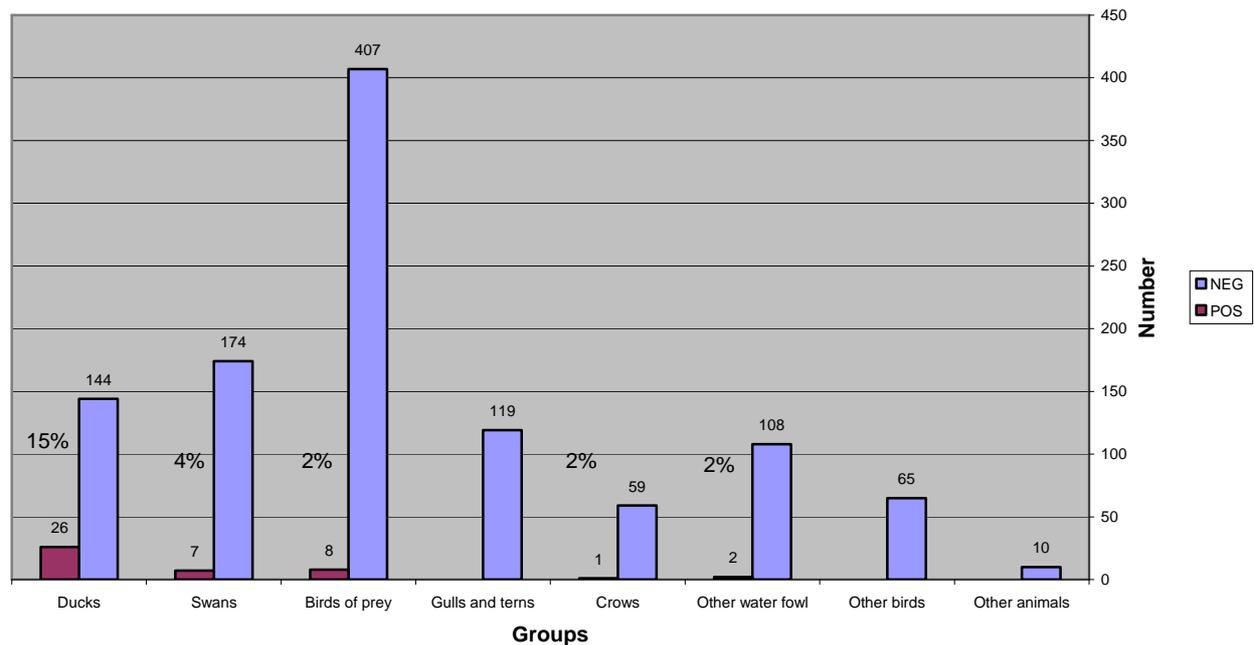


Figure 5 illustrates the total number of bird families examined and the proportional mortality rate of infected birds among each family. The highest number of birds examined was birds of prey.

¹ This figure is subject to some uncertainty, because some birds from known HP H5N1 infected areas were not examined, and due to the restrictions on dead wild birds for examination issued by the Danish Veterinary and Food Administration.

3.1.2. Surveillance of live wild birds

As a part of a continuous surveillance of wild birds since 2003, additionally 1112 samples of fresh droppings of wild birds have been analysed by PCR in spring 2006 (Table 3). These samples were collected ultimo February to the middle of June. All samples were negative for HP H5N1, except one pool of samples from five herring gulls (*Larus argentatus*) collected near the town Bisserup in the County of Storstrøm, which was found positive. The gulls had not shown any clinical signs at the time the samples were conducted. The samples are also tested for other serotypes of the AI virus, but these results are not available before the end of March 2007.

Table 3

Wild bird droppings examined from the end of February till the middle of June 2006. Bird droppings found on the same location and from the same species were pooled before analysis. Distribution by species is shown in the Appendix.

Group	Number of bird droppings
Swans	50
Goose	457
Ducks	225
Gulls	275
Other water fowl	105
Total	1112

Around the Danish HPAI outbreak in the County of Funen, a further 1007 samples of fresh droppings from wild birds were collected from 88 locations from 31 May 2006 till 26 June 2006 (Table 4).

Table 4

Wild bird droppings examined after and around the Danish HPAI outbreak. Bird droppings found on the same location and from the same species were pooled before analysis. Distribution by species is shown in the Appendix. All samples were negative for HP H5N1.

Group	Number of bird droppings
Swans	52
Birds of prey	21
Ducks	77
Gulls	760
Other water fowl	2
Other birds	93
Crows	2
Total	1007

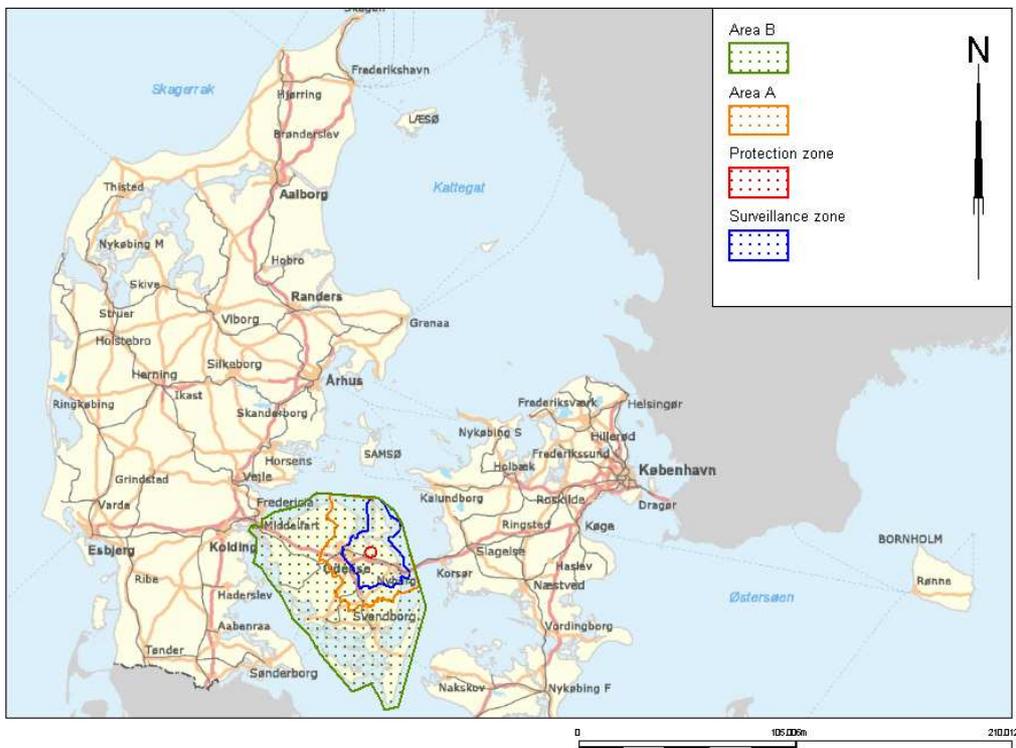
3.2 Description of the outbreak in a back-yard herd

The outbreak of HPAI in poultry in Denmark was confirmed on 18 May 2006 near the town Kerteminde in the County of Funen. The outbreak occurred in a back-yard poultry holding with 102 animals (51 chickens, 41 ducks, 5 geese, 3 guinea fowls and 2 peacocks) of which 47 had died. Furthermore, a magpie (*Pica pica*) found dead on the holding was diagnosed with HP H5N1 on the 29 May 2006. Protection and surveillance zones as well as A and B areas were immediately established on 18 May 2006 on the basis of the positive PCR result according to the Commissions Decision 2006/135/EC (Figure 6).

Figure 6

Zones and areas established on 18 May 2006 due to an outbreak of HPAI H5N1 in a backyard poultry flock.

**Status of Denmark, Avian influenza in poultry
Area A and B, protection and surveillance zone 18-05-2006**



The holding was situated close to a minor forest area and a stream. The birds had not been held in net-covered fencings in order to avoid contact with wild birds, as had been required by regulation introduced on 24 February 2006. The birds had been fed under roof, but water had been provided in the open. Also geese, ducks and poultry had not been separated. The holding was situated in a previously existing surveillance zone established due to the finding of a positive common buzzard in Ullerslev in the County of Funen on the 4 April 2006. However, the owner had not identified himself in accordance with Order No. 316 of 19 April 2006 to be a back-yard flock owner within the zone.

The birds were kept on three separated sites. The infection occurred in one of these sites, where 47 out of 52 birds died during the period 3 - 12 May (Figure 7 and 8). The owner did not call the official veterinarian, although there was a strong indication of infection with an epidemic disease. On 14 May, the owner sent three birds for examination at the National Reference Laboratory at the Danish Institute for Food and Veterinary Research, and these all tested positive for HP H5N1 by PCR. Among the 47 dead animals were 14 ducks, 29 hens, two guinea fowls and two peacocks. The five surviving birds were all ducks, and only one of these had shown clinical symptoms. The owner reported, that all sick birds had swollen and erythematous heads, ocular discharge, yellow-green diarrhoea and were depressed.

Figure 7
Timeline for AI infection in the herd. The possible infection window was estimated based on a typical incubation period of 2-5 days.

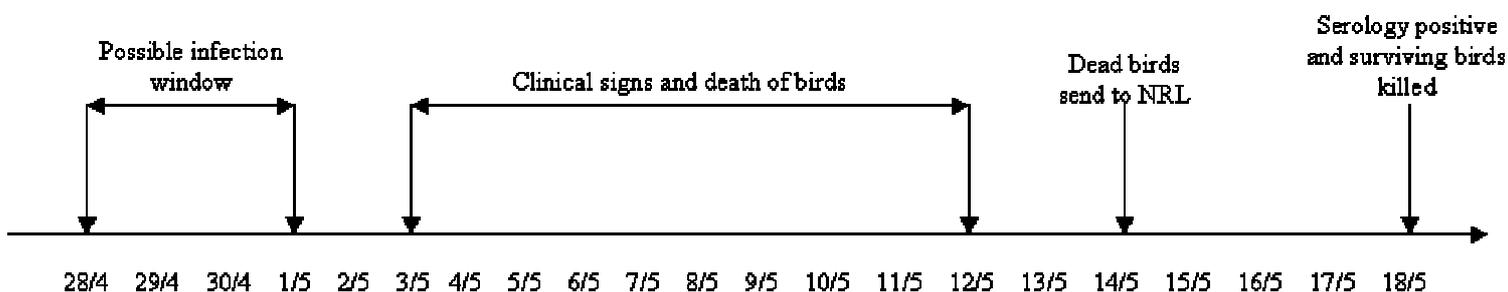
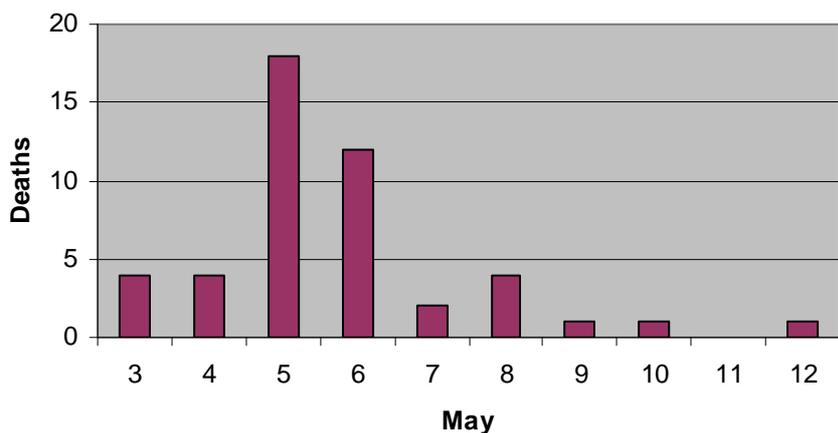


Figure 8
Daily mortality in the H5N1 affected back-yard flock showing the epidemic character of the mortality (data provided by the owner of the herd)



The owner had instituted bio-security measures such as change of boots and clothing, when the mortality had started. All birds on the holding that were killed after the positive diagnosis were tested by PCR, and only birds from the site with mortality where infected. The birds on the other two sites were not infected, indicating that the implemented bio-security measures may have been effective.

There had been no movements of birds in or out of the herd during the last 21 days before the clinical signs and mortality occurred. Four persons had, however, bought eggs for consumption at the infected herd. These persons were contacted for treatment with Oseltamivir phosphate (Tamiflu®, Roche). The owner and his wife were treated with Oseltamivir phosphate (Tamiflu®, Roche).

One small commercial layer flock and 43 back-yard flocks were located within the protection zone. In total, 27 commercial poultry herds and 448 back-yard flocks were located in the surveillance zone.

All herds within the protection zone were visited at least once by veterinarians from the regional veterinary and food administration. Commercial herds within the surveillance zone were visited while the owners of the back-yard flocks were contacted by telephone and instructed in the implementation of bio-security measures and their duty to notify mortality in their stock to the veterinary authorities. Following this screening, the zones were lifted on 30 June 2006.

The virus was of the same type as the one that had previously been detected in wild birds in the area. It was concluded, that the source of infection most likely was wild birds, possibly transmitted by direct contact. However, no direct evidence exists for this hypothesis.

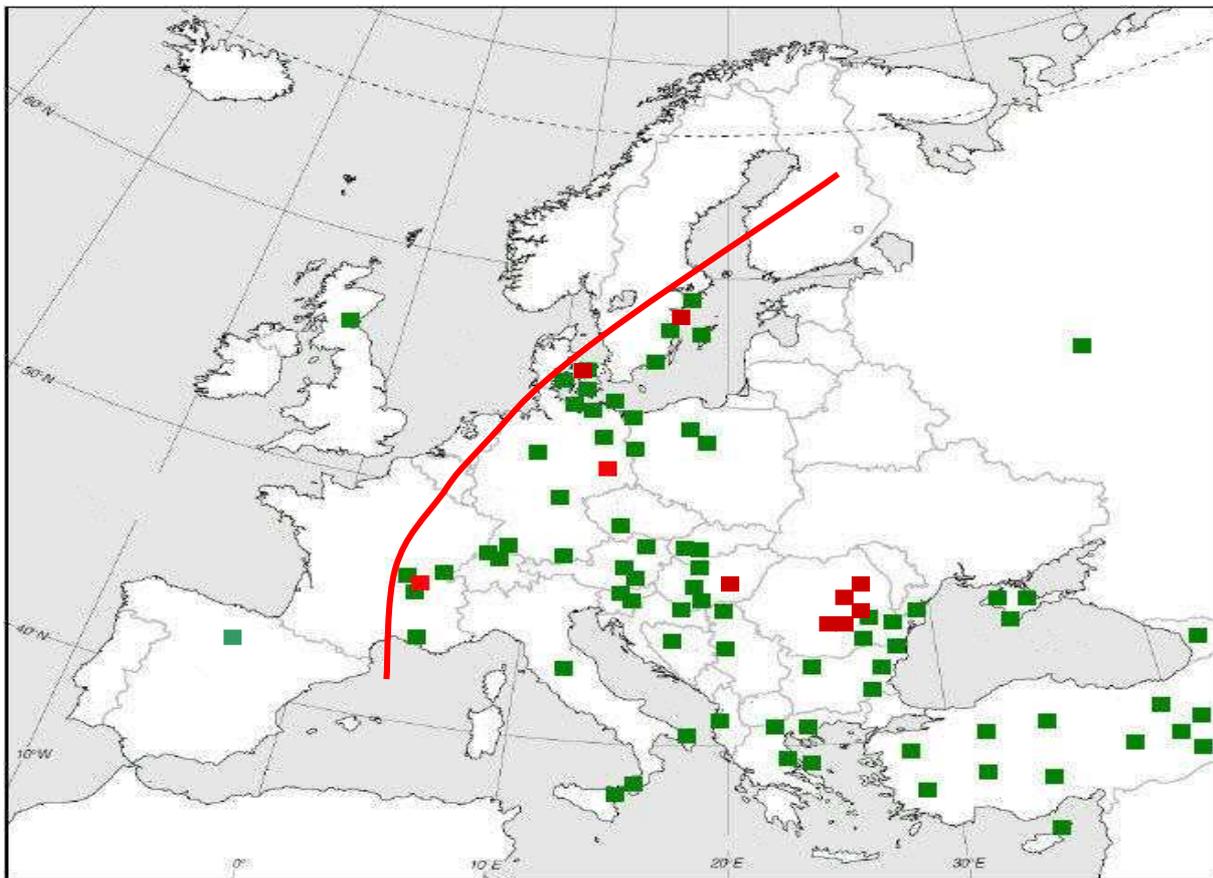
This outbreak did not cause any secondary outbreaks, and reoccurrence of the disease has not been observed since then (March, 2007).

3.3 Description of the spread of the disease

H5N1 in wild birds in Denmark was found in areas where large numbers of waterbirds normally occur during winter and early spring. However, the sequential spread of the disease did not follow the most prevalent bird migratory route – north-south – but a more east-west bound pattern. It is hypothesized, that the extreme climatic conditions with very low winter temperatures in the east European area may have forced many birds normally overwintering in open water in westerly direction, as the waters in east became ice covered. The H5N1 infection occurred below a straight line through Europe as seen on Figure 9. This apparently illustrates the western boundary for the east-west wild bird migration. An alternative explanation could be that the virus had been circulating within the Baltic area for some months before it was detected at Rügen (Brown, 2006)

Figure 9

AI front in Europe. Red dots are cases in poultry, while green dots are cases in wild birds.



Many wild bird species overwintering in Denmark migrate to breeding areas in Norway, Sweden, Finland, Russia and the Baltic area in early spring. It could therefore be expected, that infected wild birds would have been found in these areas during the summer months, but this has not happened. However, a possible explanation could be, that the findings was in fact the end of an epidemic, and that HP H5N1 failed to establish in wild birds. This explanation is supported by the figures in Figure 3 and 11. Another explanation could be that the birds breed in very remote areas, where dead birds for sampling will not be found.

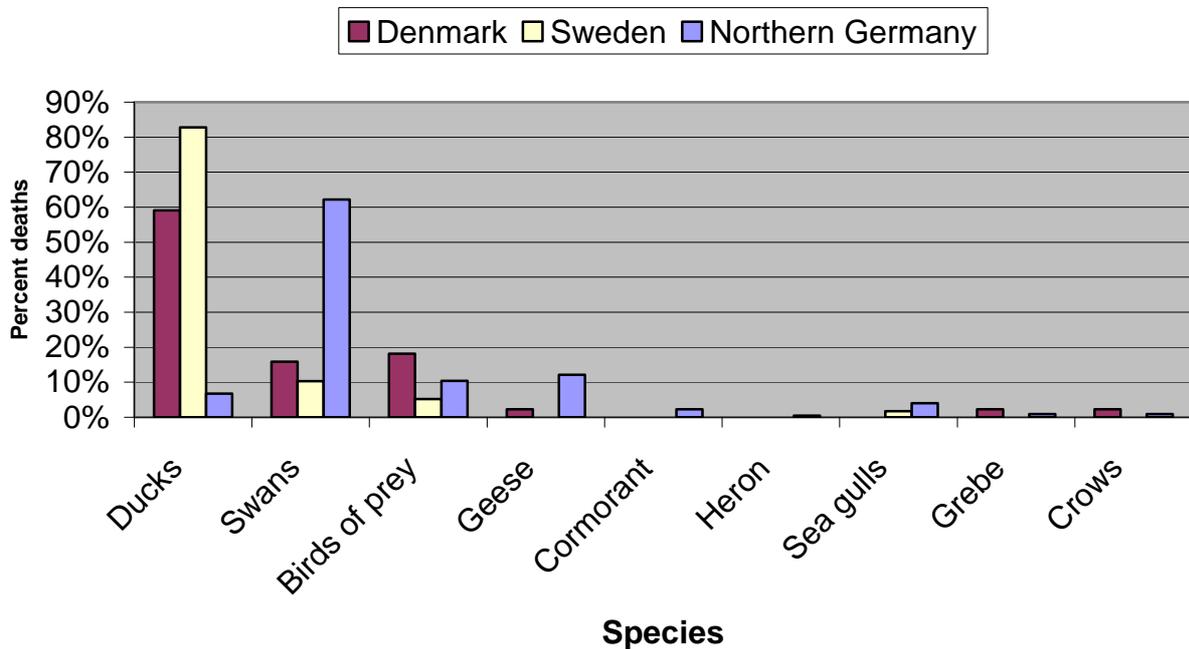
In Germany, 331 wild birds have been found infected with HP H5N1 (European Animal Disease Notification System). Of these, 222 were found in the Länder Schleswig-Holstein and Mecklenburg-Vorpommern, which are lying just south of Denmark. The distribution of species infected in these German Länder was very different from the distribution in Denmark as illustrated in Table 5 and Figure 10. In Germany, swans were the main species to be infected, whereas ducks were the main species in Denmark. These results may, however, be biased by the fact that infected tufted ducks in Denmark were found in pools of more than one infected bird (2, 3, 5 and 9), whereas this was not the case for swans. On the other hand, in Germany, infected swans were found in large pools, whereas this was not the case for tufted ducks. Furthermore, in Germany, all wild birds found sick or dead were examined regardless of how many birds were found at the same time and location. Therefore, the Danish and German figures cannot be directly compared.

In Sweden, 58 wild birds have been found infected with HP H5N1 through the passive surveillance (National Veterinary Institute). The distribution of species infected in Sweden was comparable to the distribution of species in Denmark.

Table 5
Cases of HPAI in wild birds in 2006 in the two German Länder Schleswig-Holstein and Mecklenburg-Vorpommern and in Sweden distributed by species groups.

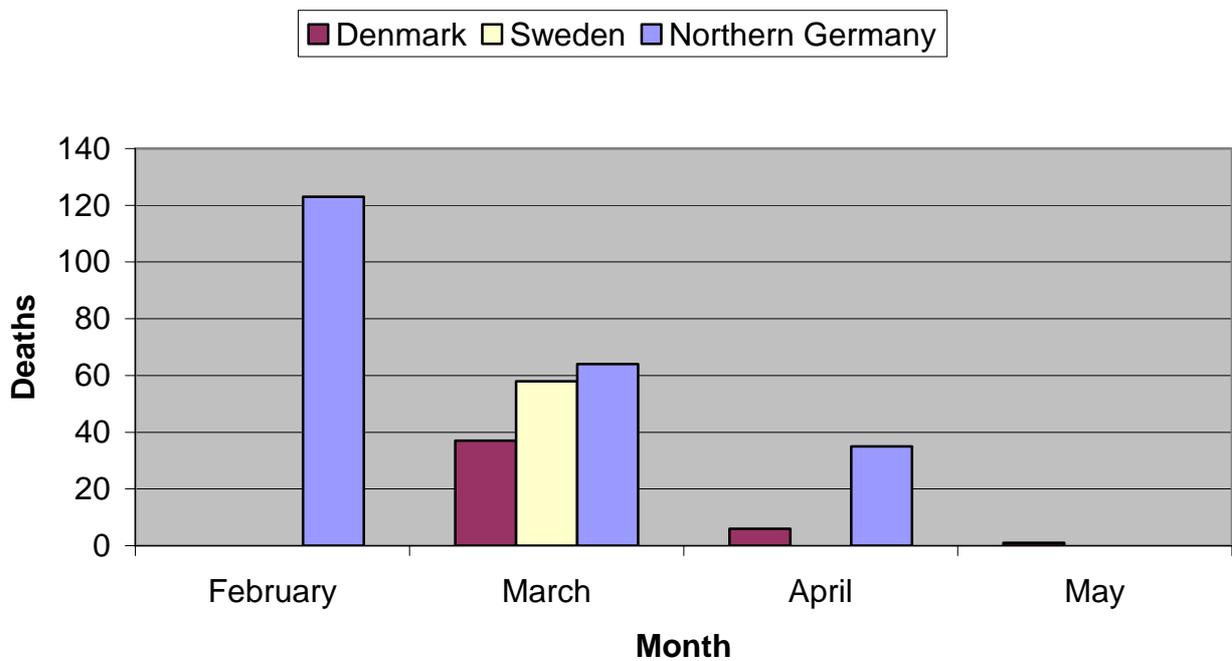
Species/species groups	North Germany		Sweden		
	Number of cases	Proportion	Number of cases	Number of tested birds	Proportional mortality rate
Ducks	15	7%	48	230	21%
Swans	138	62%	6	58	10%
Birds of prey	23	10%	3	74	4%
Other water fowl	35	16%	0	85	-
Gulls and terns	9	4%	1	60	2%
Crows	2	1%	0	21	-
Total	222	100%	58	528	11%

Figure 10
Comparing the proportion of cases of HPAI H5N1 in wild birds in Northern Germany, Sweden and Denmark distributed by species groups.



The detection of HP H5N1 occurred about a month later in Denmark compared to northern Germany as illustrated on Figure 11. However, infected birds in Sweden were only found in March.

Figure 11
Monthly numbers of HP H5N1 infected birds in Northern Germany, Sweden and Denmark.



According to the Community Reference Laboratory, Poland examined 1974 wild birds during the

period 1st February – 31st May 2006, and 70 of these were found infected with HPAI (Proportional Mortality Rate 4%). Other countries in the Baltic region tested the following number of wild birds: Estonia 151 birds, Finland 440 birds, Latvia 139 birds and Lithuania 574 birds, and none of these were found positive.

3.4 Surveillance of poultry and game birds for restocking

Surveillance of poultry and game birds for restocking was initiated in Denmark in 2003, but in February 2006, this surveillance was intensified. The surveillance is based on serology. PCR testing is only used for ducklings or chickens from game birds. Furthermore, PCR testing is used in case of a positive serological result to confirm whether virus is circulating in the herd. Only herds with more than 100 birds are included in the surveillance. Breeder flocks are tested once a year, pullets before release to egg-production, outdoors layers four times a year and outdoors slaughter poultry before slaughter. In addition, indoor turkeys are tested before slaughter. Furthermore, in risk areas breeder flocks and indoor layers are tested twice a year. Risk areas in Denmark in connection with the surveillance programme are defined as areas three km from the coastal line and around large lakes. Herds with game birds for restocking are tested four times a year during the season from February till August. When poultry and game birds are traded, they shall be accompanied by a certificate stating that the herd has been tested within the three preceding months.

Table 6
Results of the serological surveillance for AI subtypes H5 and H7 (17/2 – 30/9 2006) showing number of flocks tested. Serological flock prevalence 2,0%.

Group	Number of flocks	Number positive H5	Number positive H7	Number positive H5 and H7	Total number positive	Number positive in virological tests
Ducks/mallards, geese	30	4 (13,33 %)	2 (6,67 %)	1 (3,33 %)	7 (23,33%)	3 (10%)
Hens	438	1 (0,23 %)	2 (0,46 %)	0	3 (0,69%)	0
Turkeys	14	0	0	0	0	0
Game birds (partridge and pheasants)	129	0	0	0	0	0
Total	611	5 (0,82 %)	4 (0,65 %)	1 (0,16 %)	10 (1,63%)	3 (0,5%)

Nearly 2% of the herds examined from February till September through this surveillance were found positive in serological tests. Further testing for circulating virus in these herds resulted in three herds with game birds for restocking found infected with LPAI H5N2 and H5N3 in June and July 2006. These three herds were all killed. In two of these herds, the infection was thought to originate from contact with wild birds. In the third herd, the infection most likely originated from indirect contact with one of the other infected herds. There were no clinical signs in any of these herds, and in all three herds mallards were carrying the infection. In all cases, restriction zones were immediately established on the basis of the positive PCR result according to Council Directive 2005/94/EC. The zones were lifted following an extended screening of poultry within the zones.

Table 7
Findings of LPAI H5 in three holdings with game birds for restocking

Place	Date of confirmation	Serotype	Number of animals in herd	Zone established	Zone lifted	Source of infection
Funen County	2 June	LPAI H5N2	19.750 mallard ducklings 1606 goslings 1599 domestic ducklings	2 June	30 June	Probably contact with wild birds
Viborg County	5 July	LPAI H5N3	2679 mallard breeders 5997 mallard ducklings 140 geese 9570 pheasants 2973 partridges 50 rock partridges 99 redlegged partridges 147 doves 5 swans 117 exotic ducks 152 exotic geese 48 exotic pheasants 98 hens	5 July	13 November	Probably contact with wild birds
Funen County	19 July	LPAI H5N3	7001 ducklings	19 July	31 August	Most likely indirect contact to the holding in Viborg County

3.5 Serological methods and examination

The National Veterinary Institute, Hangevej 2, DK-8200 Aarhus N, Denmark is the National Reference Laboratory (NRL) for the diagnosis of AI. Serological tests are performed at the National Veterinary Institute, Bülowsvej 27, DK-1790 Copenhagen V.

The serological tests were carried out using haemagglutination inhibition test (HI test) in accordance with Council Directive 92/40/EC. The antigens and control sera were received from the Community Reference Laboratory. Using four HA units of antigen in the tests, sera with titres equal to or above 16 (4 log₂) were considered positive.

The virus strains Ostrich/ Denmark/ 72420/96 (H5N2) and Turkey/ England/ 647/77 (H7N7) were used as antigen in the initial test. Samples that were positive in tests with the initial antigen were subjected to a further confirmatory test with the recommended strain for the specific H-subtype, Duck/ Denmark/ 64650/03 (H5N7) and African Starling/983/79 (H7N1), respectively. A serum sample was considered positive only if HI titres were equal to or above 16 with both antigens of the same subtype.

3.6 Virological methods and examination

The National Veterinary Institute, Hangevej 2, DK-8200 Aarhus N, Denmark as the NRL, performed the diagnostic work. The NRL, Department for Poultry, Fish and Fur Animals, Aarhus, performs all virological diagnostic analyses for AI in Denmark.

The primary diagnostic procedures are based on RT-PCR methods, but do also include virus isolation by inoculation in SPF embryonated eggs. The RT-PCR protocols applied at the Danish Institute for Food and Veterinary Research have been tested as a part of an EU-project (AVIFLU) and they are subsequently recommend by the EU-reference laboratory, Weybridge, UK. The methods conform to the methods required by Council Directive 2005/94/EC.

The specific RT-PCR analysis for general influenza A applied primers are specific to the viral matrix (M) gene or the nucleoprotein (NP) gene. The H5 and H7 specific analyses apply primers, which only detects the viral haemagglutinin (HA) gene of the H5 and H7 subtypes, respectively.

Analyses for general influenza virus (M-gene) and specific H5 subtype RT-PCR detection are performed on all samples received for influenza diagnosis.

- 1) If a sample is tested positive in general and negative for H5, a supplementary H7 analysis is performed
- 2) If a sample is detected positive for H5 or H7 the RT-PCR product is sequenced for confirmation of the H5 or H7 subtype and for characterisation of the virus in terms of pathogenicity.
- 3) If a sample is positive in the M-gene RT-PCR and negative for both H5 and H7 types, a RT-PCR analysis applying detection of the nucleoprotein (NP) is performed for influenza A virus confirmation.
- 4) If a sample is positive by the M-gene RT-PCR the sample is inoculated in SPF embryonated eggs.

Virus cultivation utilise 8-10 days old embryonated SPF eggs, which are inoculated by the allantoic route. The eggs are incubated for one week and the harvest of allantoic fluid is tested for presence of haemagglutinating viruses. Agglutinating viruses are subtyped by HI test. In addition, identification RT-PCR and sequencing is carried out in accordance with the above description.

A final characterisation of a virus isolate is done by conventional neuraminidase test (N-typing). In addition, a N-1 specific RT-PCR method may be applied to samples collected either directly from sick or dead birds or harvested from inoculated SPF embryonated eggs.

4. Disease Control

4.1 Legislative matters

During the findings of HP H5N1 a series of Orders were issued in order to regulate export and national transport of poultry, gatherings of birds, markets and exhibitions.

4.2 Crises management structure

On 3 March 2006, after several findings of infected wild birds on the island of Rügen in Germany close to the Danish border and a finding of infected tufted ducks in Sweden, a Crisis Management Structure was established in order to optimise the coordination of the efforts to control an outbreak of HPAI. The Central Coordination Centre coordinated the activities in the four task force groups: Epidemiology and Eradication, Information and Media, Transport and Movements and Documentation and Log (Appendix II).

The Epidemiology and Eradication task force group coordinated the epidemiological investigations, the sampling of diagnostic material for testing, the eradication of infected poultry and the disinfection of the infected holding and measures established in connection with the findings of H5N1 infected wild birds. The work was conducted in close collaboration with the local Animal Health Unit in the affected regions. Moreover, the Epidemiology and Eradication task force group was responsible for imposing protection and surveillance zones around the infected holding and areas, where infected wild birds were found. A central GIS service was set up to provide maps and geographic information for the local Animal Health Unit. The location of established protection and surveillance zones were presented at the website of the Danish Veterinary and Food Administration. Due to this service, the poultry industry, poultry owners and the Danish Veterinary and Food Administration personnel could locate any site in relation to current protection and surveillance zones.

The Information and Media task force group continuously updated the European Commission on findings and on the outbreak in the back-yard herd and informed the OIE. A notification to the OIE took place as soon as the first official HP H5 was confirmed (within 24 hours). Moreover, the Food and Agricultural Counsellors at Danish embassies were regularly updated on the HPAI situation with the objective to inform the Veterinary Administrations in Third Countries. The task force group was also responsible for the administration of the Animal Disease Notification System (ADNS) and the update of the Danish Veterinary and Food Administration homepage in both Danish and English. This task force group was also responsible for giving information to the Ministry of Family and Consumer Affairs and the Parliament, the Danish Poultry Industry, other departments in the Danish Veterinary and Food Administration, the veterinary practitioners and the media. Furthermore, a special hot-line was established to answer questions from the citizens. There was a close cooperation with the Danish police and the Danish Health Services concerning the public health issue.

The Transport and Movement task force group was responsible for the administration of the control of transport and movement, including export of animals and products according to EU legislation and implementing Danish orders with ban on movement of poultry and products, to further reduce the risk of transmission of the infection. The task force coordinated the licensed transport and movement of animals and products within or between protection and surveillance zones in cooperation with the local Animal Health Units.

The Documentation and Log group was responsible for building up an unambiguous common log system, which could be read from the Central Coordination Centre as well as from the local Animal Health Unit. They collected the necessary technical information from the local Animal Health Units and coordinated this, and all relevant information was filed.

4.3 Measures in case of suspicion

According to Order No. 921 of 10 November 1994 with subsequent changes, the owner of poultry or other birds, suspected to be infected with AI virus is obliged to call a veterinary practitioner. The veterinary practitioner is obliged to notify the local Animal Health Unit if AI infection is suspected and to take out samples for serological and virological examinations according to the instructions by the local Animal Health Unit. The local Animal Health Unit imposes movement restrictions on the farm and on possible contact farms. In Denmark, the veterinary officer from the local Animal Health Unit shall visit the herd within three hours, and samples shall be sent to the diagnostic laboratory within eight hours after being notified of the suspicion. The owner will get reimbursement for the animals from the central authorities in case the suspicion is confirmed. The central authority also pays for cleaning and disinfection and pays 20% of the owner's loss of profits.

Furthermore, an early warning system has been set up according to Order No. 62 of 17 March 2006, whereby owners of poultry or other birds are obliged to notify the local Animal Health Unit in case of decreasing food or water intake, a decreasing egg production or an increased mortality. In these cases the herds are not put under movement restrictions.

In case the general public find dead birds in nature they can contact the local Animal Health Unit. If AI is suspected the birds are under appropriate safety measures collected by personnel from the Danish Emergency management agency and brought to the laboratory for virological examination. In Denmark, the wild birds shall be collected on the same day or if notified after 1 pm on the following day.

4.4 Measures in case of confirmed outbreaks

In the confirmed outbreak of HP H5N1 in a poultry herd, measures according to Council Directive 92/40/EC were imposed. In the confirmed outbreaks of LPAI, measures according to Council Directive 2005/94/EC were imposed. All poultry on the infected farms were killed and disposed by rendering, followed by cleaning and disinfection of the farm. In Denmark, the animals on the infected farms shall be killed within 24 hours after diagnosis, and the preliminary cleaning and disinfection shall be approved within two – eight days. The Danish Veterinary and Food Administration have a Royal ordinance to issue Orders on their homepage, which are immediately valid, in case of outbreaks of infectious animal diseases.

4.5 Killing

The birds in the infected herds were killed by injection of Pentobarbital sodium as an intraperitoneal injection of a 30% solution. The dose was 5 – 20 ml according to the size of the bird. After the injection the poultry were left without any disturbance until death had supervened. Game birds like pheasants and partridges were killed in CO₂ filled containers.

4.6 Cleaning and disinfection

The requirements concerning cleaning and disinfection of the HP H5N1 infected holding were provided by the provisions of article 11 and Annex II of Council Directive 92/40/EC, whereas these requirements for the LP infected holdings were provided by the provisions of article 48 and Annex VI of Council Directive 2005/94/EC.

As described below the key points as regard the cleaning and disinfection have been:

- The procedure for cleaning and disinfection as described in Annex II of Council Directive 92/40/EC or Annex VI of Council Directive 2005/94/EC
- The cleaning and disinfection operations were carried out under official supervision
- The Regional Veterinary Officer carried out the approval of the preliminary and of the final cleaning and disinfection.

As soon as the killed poultry was transported to an approved rendering plant the preliminary cleaning and disinfection was initiated.

In case of repopulation of the premises, this cannot happen before 21 days after the final cleaning and disinfection, and then the birds has to be examined closely before and after the repopulation. The back-yard herd, which experienced an outbreak of HP H5N1 in Denmark in 2006 has not been repopulated (January, 2007).

4.7 Measures taken in protection and surveillance zones

Protection (at least 3 km) and surveillance (at least 10 km) zones around findings of wild birds with HP H5N1 were established according to Commission decision 2006/115/EC. Protection and surveillance zones around the outbreak in a back-yard herd were established according to Council Directive 92/40/EC, while A and B areas around this outbreak were established according to Commission decision 2006/135/EC. Restriction zones around herds found infected with LPAI were established according to Council Directive 2005/94/EC.

When zones and areas where established, a complete stand-still for all poultry and game birds including eggs where ordered. Letters informing all owners of poultry or game birds for restocking within these zones and areas, where immediately sent, when zones and areas where established. Authorizations to move poultry or game birds from these areas where given from the local animal health unit based on a thorough risk assessment in each case.

4.8 Registration of poultry flocks

According to Danish legislation, commercial flocks of poultry are registered in the Central Husbandry Register. Commercial flocks in protection and surveillance zones were identified via the database.

Order No. 316 of 19 April 2006, issued due to the HPAI situation in wild birds in Denmark, provided the legal power to require owners of hobby flocks to identify themselves, if they were located in risk areas. In the same way, order No. 9395 of 29 June 2006, issued due to the HPAI outbreak in a back-yard flock on Funen, provided the legal power to require owners of hobby flocks

to identify themselves, if they were located in risk areas around the finding place of a H5N1 infected wild bird or an infected poultry flock.

The specific risk areas (zones) during the epidemic were: The Counties of Funen, Sønderjylland, Storstrøm, and parts of West Zealand, Roskilde and Frederiksborg Counties.

Owners of hobby flocks had the possibility to register themselves via the DVFA homepage or by phone/mail to the local Animal Health Unit. Announcements were made in the press in connection to all findings of infected wild birds calling on all owners of hobby flocks within the zones to register themselves.

A total of about 3400 hobby flocks were registered on the basis of these efforts.

4.9 Inspections and screening in protection and surveillance zones

Protection and surveillance zones established due to HP H5N1 in wild birds

According to the Commissions Decision 2006/115/EC, a protection zone established due to findings of HP H5N1 in wild birds can be lifted, 21 days after the bird was received at the laboratory for analysis. A surveillance zone can be lifted 30 days after the bird was received at the laboratory for analysis.

All commercial poultry herds and game bird breeders in the protection and surveillance zones were visited once or twice. Back-yard flocks in the protection zones were visited at least once, if there were ducks in the herd. Otherwise, they were contacted by phone and visited only in case of suspicion. All game bird breeders with mallards and herds with domestic ducks in the protection zones, which had not held these in net-covered enclosures, were sampled and tested serologically. All back-yard herds with mallards or domestic ducks in the protection zones were visited, and those, which had not held the birds under net-cover, were sampled and tested serologically as well.

Protection and surveillance zones established due to HPAI H5N1 in a back-yard holding

According to Council Directive 92/40/EC, a protection zone established due to findings of HP H5N1 in poultry herds or herds with game birds for restocking can be lifted at the earliest, when 21 days have elapsed since the preliminary cleaning and disinfection of the infected herd. Furthermore, all poultry herds within the zone including back-yard herds shall be visited before lifting the zone. A surveillance zone can be lifted, when 30 days have elapsed since the preliminary cleaning and disinfection of the infected herd.

All poultry herds, herds with game birds for restocking and back-yard herds were visited once or twice in the protection zones. All herds with mallards for restocking and herds with domestic ducks, which had not been held in net-covered enclosures, were sampled and tested serologically. All back-yard herds with mallards or domestic ducks were visited, and those, which had not been held in net-covered enclosures, were sampled and tested serologically as well. Commercial herds within the surveillance zone were visited, while the owners of the back-yard flocks were contacted by telephone and instructed in the implementation of bio-security measures and their duty to notify mortality in their stock to the veterinary authorities.

Restriction zones established due to LPAI in herds with game birds for restocking

According to Council Directive 2005/94/EC, a restriction zone established due to findings of LPAI in poultry herds or herds with game birds for restocking can be lifted, when 21 days have elapsed

since the preliminary cleaning and disinfection of the infected herd. Furthermore, all commercial poultry herds and herds with game birds for restocking within the zones shall be visited before lifting the zones.

All commercial poultry herds and herds with game birds for restocking were visited once or twice in the restriction zones. All herds with mallards for restocking and herds with domestic ducks, which had not held these under net-cover, were sampled and tested serologically.

4.10 Screening results

In April – May a total of 165 poultry holdings were visited at least once, 833 back-yard herds were contacted and 58 back-yard herds with ducks were visited. In total, 11 (19%) of the 58 herds with ducks had not been covered in order to avoid contact with wild birds, and they were therefore tested serologically. All these tests were negative.

5. Conclusions

The Danish epidemic was foreseen due to prior findings in Germany and Sweden in the areas around the Baltic Sea. The Danish veterinary authorities were, therefore, well prepared for the findings of wild birds infected with HP H5N1. Restrictions on all poultry and game birds to be kept in net-covered enclosures – except mallard ducklings – in order to avoid contact with wild birds were immediately imposed. The outbreak in a back-yard holding, where the birds had not been net-covered according to these provisions, justified the restrictions.

The serological surveillance programme has shown, that LPAI in fact is circulating in the population mainly in mallards acting as asymptomatic carriers of AI. The aim of the surveillance is to find and eradicate any AI-virus circulating in the population by killing of all birds in the herds found infected with LPAI.

The findings of HPAI H5N1 in wild birds disappeared very suddenly in May, probably because the infection failed to establish in wild birds, or due to the spring migration. It may be expected, that the infection could reappear in wild birds during the migratory periods, which takes place during the periods 15 September to 15 November in the autumn and 1 March to 30 April in the spring. Therefore, all poultry and game birds for restocking shall be held in net-covered fencings during these periods. All year, poultry and game birds for restocking shall be given food and water under roof, and geese, ducks and poultry shall be separated.

References

Brown, Ian; 2006. Personal communication.

European Food Safety Authority 2005. SCIENTIFIC REPORT, Animal health and welfare aspects of Avian Influenza, Adopted on 13/14 September 2005.

European Food Safety Authority 2005. Scientific Opinion on Animal health and welfare aspects of Avian Influenza, EFSA-Q-2004-075.

National Veterinary Institute, <http://www.sva.se/>

World Health Organization, 2005. Responding to the avian influenza pandemic threat, Recommended strategic actions.

World Health Organization: Epidemic and Pandemic Alert and Response (EPR), Confirmed Human Case of Avian Influenza A (H5N1), 12 March 2007.

[Cumulative Number of Confirmed Human Cases of Avian Influenza A/\(H5N1\) Reported to WHO](#)

Appendix I

Table 8
Number of dead birds and mammals examined from January to June 2006 distributed by species.

Species	Latin name	Result		
		Negative	Positive	Total
Arctic tern	<i>Sterna paradisaea</i>	1		1
Barn owl	<i>Tyto alba</i>	4		4
Barnacle goose	<i>Branta leucopsis</i>	1		1
Bewick's swan	<i>Cygnus columbianus</i>	1		1
Blackbird	<i>Turdus merula</i>	13		13
Black-headed gull	<i>Larus ridibundus</i>	17		17
Cat	<i>Felis silvestris catus</i>	7		7
Common buzzard	<i>Buteo buteo</i>	214	6	220
Common scoter	<i>Melanitta nigra</i>	6		6
Common tern	<i>Sterna hirundo</i>	7		7
Coot	<i>Fulica atra</i>	24		24
Sparrowhawk	<i>Accipiter nisus</i>	128		128
Cormorant	<i>Phalacrocorax carbo</i>	39		39
Domestic pigeon	Columbidae	6		6
Duck	Anatini	2		2
Eider	<i>Somateria mollissima</i>	93		93
Fieldfare	<i>Turdus pilaris</i>	3		3
Fowls	<i>Gallus gallus domesticus</i>	5		5
Golden eagle	<i>Aquila chrysaetos</i>	1		1
Goldeneye	<i>Bucephala clangula</i>	1		1
Goshawk	<i>Accipiter gentilis</i>	15		15
Great crested grebe	<i>Podiceps cristatus</i>	1	1	2
Great northern diver	<i>Gavia immer</i>	1		1
Grey heron	<i>Ardea cinerea</i>	23		23
Greylag Goose	<i>Anser anser</i>	3	1	4
Gull	<i>Larus</i>	61		61
Hare	Leporidae	1		1
Heron	<i>Ardea cinerea</i>	4		4
Herring gull	<i>Larus argentatus</i>	27		27
Hooded and carrion Crow	<i>Corvus corone</i>	12		12
Horned owl	<i>Bubo</i>	2		2
Jackdaw	<i>Corvus monedula</i>	4		4
Kestrel	<i>Falco tinnunculus</i>	21		21
Lapwing	<i>Vanellus vanellus</i>	2		2
Lark	<i>Alauda arvensis</i>	1		1
Little owl	<i>Athene noctua</i>	1		1
Long-eared owl	<i>Asio otus</i>	2		2
Long-tailed Duck	<i>Clangula hyemalis</i>	1		1
Magpie	<i>Pica pica</i>	29	1	30

Species	Latin name	Result		
		Negative	Positive	Total
Mallard	Anas platyrhynchos	24		24
Merganser	Mergus	3		3
Merlin	Falco columbarius	1		1
Mistel thrush	Turdus viscivorus	2		2
Muscovy duck	Cairina moschata	1		1
Mute swan	Cygnus olor	60	4	64
Owl	Strigidae	1		1
Oystercatcher	Haematopus ostralegus	1		1
Peregrine	Falco peregrinus		1	1
Pheasant	Phasianus colchicus	6		6
Pigeon	Columbidae	1		1
Pochard	Aythya ferina	1		1
Raven	Corvus corax	2		2
Redpoll	Carduelis flammea	1		1
Rook	Corvus frugilegus	12		12
Rough-legged buzzard	Buteo lagopus	13	1	14
Shelduck	Tadorna tadorna	1		1
Short-eared owl	Asio flammeus	2		2
Song thrush	Turdus philomelos	3		3
Spotted woodpecker	Dendrocopus	1		1
Starling	Sturnus vulgaris	1		1
Stone marten	Martes foina	2		2
Swan	Cygnus	99		99
Tawny owl	Strix aluco	1		1
Teal	Anas crecca	1		1
Tern	Sterna	6		6
Thrush	Turdus	9		9
Tufted duck	Aythya fuligula	10	26	36
Waxwing	Bombycilla garrulus	13		13
White-tailed eagle	Haliaeetus albicilla	1		1
Whooper swan	Cygnus cygnus	14	3	17
Woodcock	Scolopax rusticola	9		9
Total		1086	44	1130

Table 9
Wild bird droppings examined from the middle of January till the end of June 2006
distributed by species.

Species	Latin name	Number of bird droppings
Barnacle goose	<i>Branta leucopsis</i>	50
Black-headed gull	<i>Larus ridibundus</i>	100
Brent goose	<i>Branta bernicla bernicla</i>	78
Brent goose	<i>Branta bernicla hrota</i>	44
Common gull	<i>Larus canus</i>	75
Cormorant	<i>Phalacrocorax carbo</i>	30
Golden plover	<i>Pluvialis apricaria</i>	75
Greylag goose	<i>Anser anser</i>	110
Greylag goose/Canada goose	<i>Anser anser/Branta canadensis</i>	100
Herring gull	<i>Larus argentatus</i>	100
Mallard	<i>Anas platyrhynchos</i>	75
Mute swan	<i>Cygnus olor</i>	50
Pink-footed goose	<i>Anser brachyrhynchus</i>	75
Surface-feeding duck	<i>Anas sp.</i>	15
Teal	<i>Anas crecca</i>	60
Wigeon	<i>Anas penelope</i>	75
Total		1112

Table 10
Wild bird droppings examined after the Danish HPAI outbreak distributed by species.

Species	Latin name	Number of bird droppings	Number of localities
Barn owl	<i>Tyto alba</i>	3	1
Black-headed gull	<i>Larus ridibundus</i>	276	2
Blue tit	<i>Parus caeruleus</i>	6	1
Common gull	<i>Larus canus</i>	201	5
Coot	<i>Fulica atra</i>	2	1
Eider	<i>Somateria molissima</i>	73	6
Great tit	<i>Parus major</i>	4	1
Herring gull	<i>Larus argentatus</i>	243	7
Hooded crow	<i>Corvus corone</i>	2	1
House martin	<i>Delicon urbica</i>	40	40
House sparrow	<i>Passer domesticus</i>	10	2
Kestrel	<i>Falco tinnunculus</i>	18	6
Lesser black-backed gull	<i>Larus fuscus</i>	20	1
Mute swan	<i>Cygnus olor</i>	52	5
Sandwich tern	<i>Sterna sandvicensis</i>	20	1
Starling	<i>Sturnus vulgaris</i>	10	1
Surface-feeding duck.	<i>Anas sp.</i>	1	1
Swallow	<i>Hirundo rustica</i>	23	4
Tufted duck	<i>Aythya fuligula</i>	3	2
Total		1007	88

Appendix II

Figure 12
Plan for crisis management at central level.

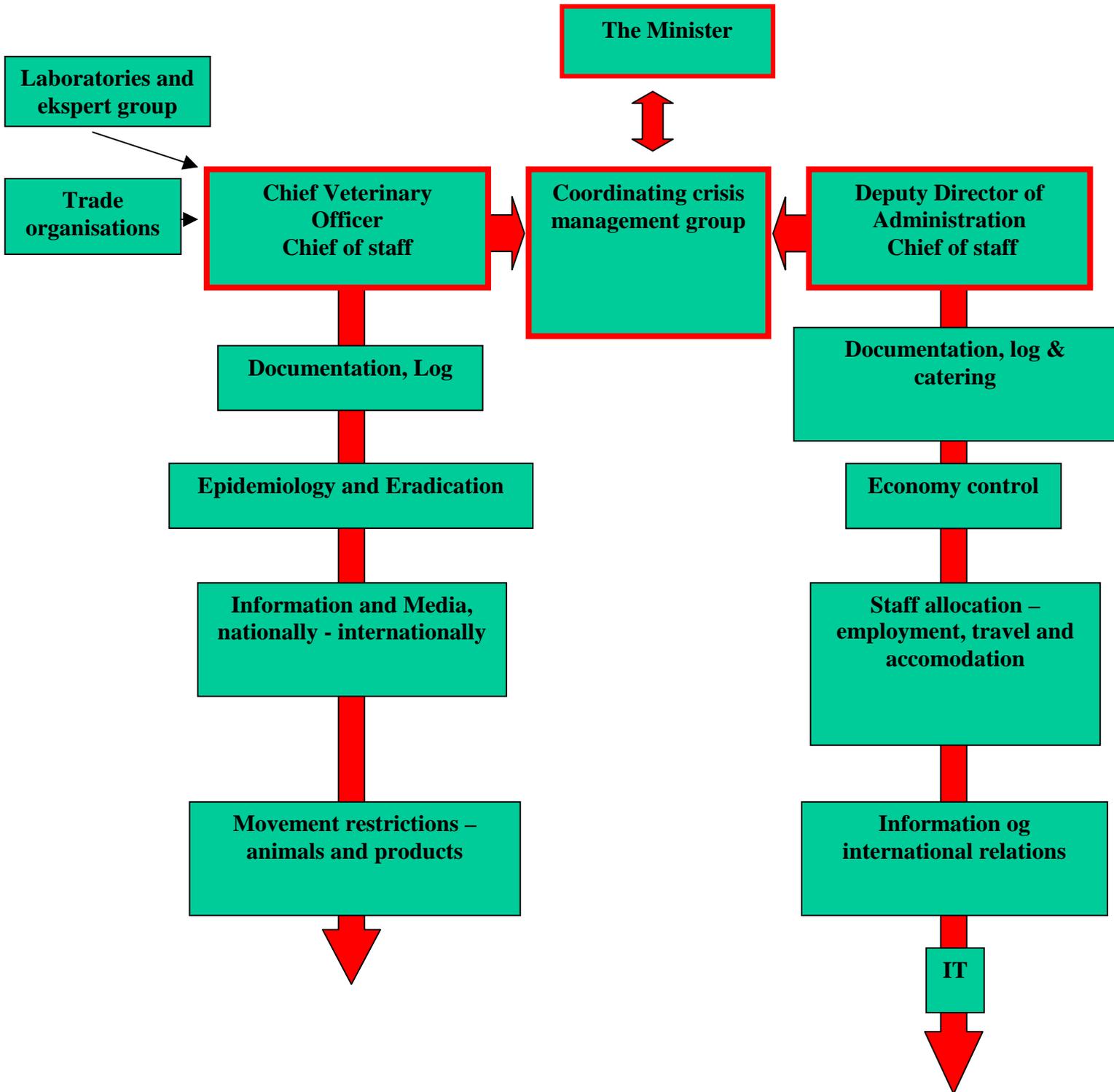
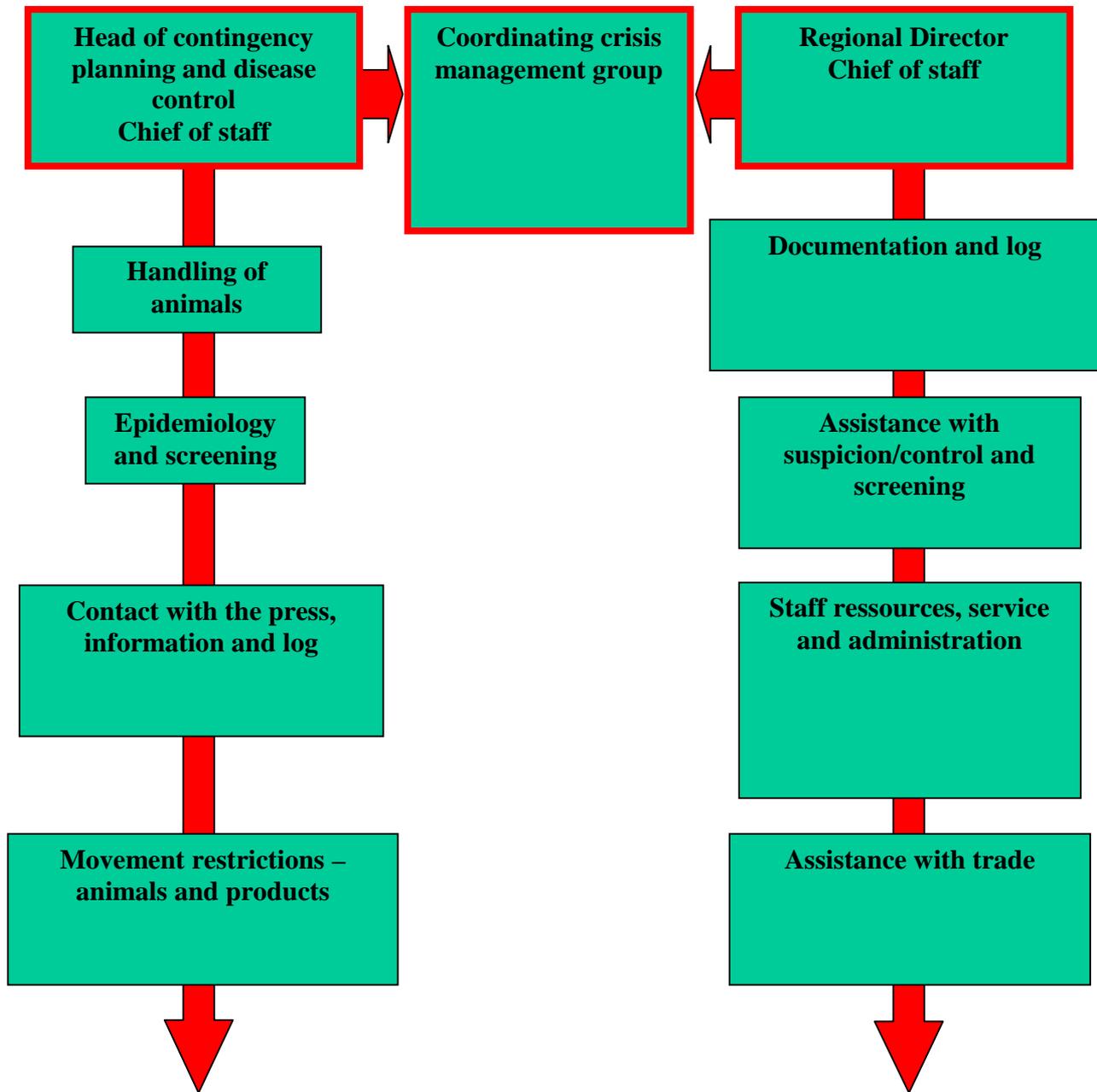


Figure 13
Plan for crisis management at regional level.



Appendix III

Table 11

All protection zones established in Denmark during spring 2006 due to detection of HPAI H5 in wild birds

Protection zone	Date established	Date lifted
Ærø	17 March	8 May
Roskilde fjord	19 March	4 May
Præstø	22 March	5 May
Drejø	24 March	8 May
Guldborgsund	25 March	1 May
Svendborg	25 March	8 May
Stubbekøbing	27 March	1 May
Nysted	27 March	1 May
Alssund	29 March	4 May
Kegnæs – ved Als	30 March	4 May
Vang – Bornholm	31 March	27 April
Skælskør	31 March	21 April
Fåborg	27 March	8 May
Fåborg, Assensvej	1 April	8 May
Bandholm	1 April	24 April
Ullerslev	7 April	8 May
Hornbæk	7 April	3 May
Gråsten	2 May	30 May

Table 12

All surveillance zones established in Denmark during spring 2006 due to detection of HPAI H5 in wild birds

Protection zone	Date established	Date lifted
Præstø	21 March	5 May
Det sydfynske øhav (Ærø)	17 March	25 March
Roskilde fjord	19 March	4 May
Guldborg	25 March	1 May
Svendborg – Det sydfynske øhav	25 March	8 May
Nysted	27 March	1 May
Stubbekøbing	27 March	5 May
Falsled	27 March	8 May
Als Sundeved	29 March	4 May
Bornholm Nord	31 March	27 April
Vest Lolland	1 April	24 April
Hornbæk	7 April	3 May
Ullerslev	7 April	8 May
Svinø	15 March	21 April
Skælskør by	31 March	24 April
Gråsten	2 May	30 May